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Award Number: DAMD17-97-1-7072

TITLE: DNA Repair and Breast Cancer Risk

PRINCIPAL INVESTIGATOR: Kathy J. Helzlsouer, M.D., M.H.S.

CONTRACTING ORGANIZATION: Johns Hopkins University
Baltimore, Maryland 21205

REPORT DATE: October 2001

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	October 2001	Final (1 Oct 97 - 30 Sep 01)

4. TITLE AND SUBTITLE
DNA Repair and Breast Cancer Risk

5. FUNDING NUMBERS
DAMD17-97-1-7072

6. AUTHOR(S)
Kathy J. Helzlsouer, M.D., M.H.S.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Johns Hopkins University
Baltimore, Maryland 21205

E-Mail: khelzlso@jhsph.edu

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT ***(Maximum 200 Words)***

Investigations of potential gene-environment interactions may improve our understanding of the etiology of breast cancer, a disease where less than 40% of cases can be attributed to known risk factors. The objectives of this proposal are to examine the association between DNA repair proficiency and breast cancer risk, and the contribut8ion of this factor to the familial clustering of breast cancer. We hypothesize that mechanisms leading to suboptimal repair of DNA damage are susceptibility factors predisposing women to breast cancer through increased sensitivity to carcinogenic damage from environmental exposures such as ionizing radiation

To evaluate this hypothesis a case-control study was conducted. Women with a personal history of breast cancer and women at increased breast cancer risk are being compared to control women for the presence of Lys/Lys 751 EPPPD genotype. We observed a strong association between presence of the Lys/Lys 751 XPD genotype and higher number of chromatid aberrations. Thus, we are assessing the XPD genotype, in place of the DNA repair proficiency cytogenetic assay which we originally planned to do. We will also evaluate the possible interaction between Lys/Lys 751 XPD genotype and ionizing radiation exposure, by stratifying the case-control data on exposure status, and assessing the relation between breast cancer risk and the genotype. Delay introduced by difficulties in assay development caused us to run out of time and funds before we could complete recruitment to a planned family study.

14. SUBJECT TERMS	15. NUMBER OF PAGES
Breast Cancer, RTP Award, XPD polymorphisms, DNA Repair	16
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

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ANNUAL REPORT YEAR 4: DNA REPAIR AND BREAST CANCER RISK STUDY

1. INTRODUCTION

We hypothesize that mechanisms leading to suboptimal repair of DNA damage are susceptibility factors predisposing women to breast cancer through increased sensitivity to carcinogenic damage from environmental exposures such as ionizing radiation. We conducted a case-control study to address the following research question:

> Do women with breast cancer and women with a family history of breast cancer more often have suboptimal repair of DNA damage compared to control women?

We originally planned to measure DNA repair proficiency using the cytogenetic assay developed at NIH by Sanford and Parshad and used in our pilot study in 1993-5. Due to problems with repeatability of the assay in our laboratory, we are instead examining specimens for common polymorphisms in genes that encode enzymes involved in DNA repair pathways. Due to delay introduced by difficulties in assay development, we ran out of time and funds before we could complete recruitment to a planned family study. As stated in our year 3 report, we will recruit additional families and family members as funding is available.

2. BODY: ANNUAL REPORT YEAR 4

Case-control and Family studies, Tasks 1-6

Task 1: Develop and produce study brochure. Finalize questionnaire and family history form.

We developed study brochures for recruiting participants and finalized the questionnaire and family history form in year 1.

Task 2: Identify and recruit eligible participants (300 cases and controls and 150 family members).

We completed recruitment to the case-control study on February 13, 2001. We recruited 95 incident breast cancer cases; 144 women at increased risk of breast cancer due to family history of breast cancer or results of breast biopsies; and 134 control women, for a total of 373 cases and controls.

The case-control study was clinic-based. We recruited among women attending clinics at the Breast Center at the Johns Hopkins Outpatient Center; women having mammograms at the the Johns Hopkins Outpatient Center; women visiting Medical Oncology and Radiation Oncology at the Johns Hopkins Oncology Center; and among employees and students of the Johns Hopkins East Baltimore Campus. The majority of our employee and student participants seek care at outpatient clinics of the Johns Hopkins Medical Institutions, and therefore are similar to clinic patients.

Cases are women with newly diagnosed breast cancer; women diagnosed with DCIS; women with a family history of breast cancer; and women who have had breast biopsies. Diagnosed cases include women diagnosed with breast cancer in the last year, or with DCIS at any time. Cases with a family history include women with at least one first-degree relative, or two second-degree relatives on the same side of the family, diagnosed with breast cancer. Cases with biopsies include women diagnosed with proliferative disease, hyperplasia, atypia, fibroadenoma, or LCIS. Controls are women with at most one second-degree relative with breast cancer on either side of the family; no cancer in first-degree relatives other than non-melanoma skin cancer; and no history of biopsy other than aspiration of a fluid-filled cyst. Women with cancers other than breast cancer, other than non-melanoma skin cancer, were excluded. All participants were age 20 or older.

Recruitment for the planned family study could not be completed. We recruited 19 family members of 16 women diagnosed with breast cancer, or with a family history of breast cancer. Pending results of the case-control study, we will recruit additional families and family members as funding is available.

Task 3: Collect questionnaire data and blood samples.

Blood samples have been collected from all participants, and more than 97% of questionnaires and family history forms were completed and returned.

Task 4: Perform DNA repair assays.

We originally planned to measure DNA repair proficiency using the cytogenetic assay developed at NIH by Sanford and Parshad (1) and used in our pilot assay in 1993-5, to examine repair of DNA damage in lymphocytes. Due to problems with repeatability of the cytogenetic assay in our laboratory, we stopped doing the assay in November of 1999. We are, instead, examining specimens for common polymorphisms in genes (> 5-10% of population) that encode enzymes involved in DNA repair pathways. The difficulties in reproducing the cytogenetic assay have been extensively detailed in previous reports and are not reviewed here.

We conducted a genotype-phenotype study using DNA from 31 women whose blood was assayed for DNA repair proficiency by the cytogenetic assay in our pilot study at NIH (2) (Appendix 1). We observed a strong association between presence of the Lys/Lys 751 XPD genotype, and higher number of chromatid aberrations in the cytogenetic assay (Table 1). Thus, we are assessing common polymorphisms in defined DNA repair pathway genes, in place of the DNA repair proficiency cytogenetic assay which we originally planned to do.

Table 1. Characteristics of Cases and Controls

	Cancer Cases (N=95)	High Risk Cases (N=144)	Controls (N=134)
Age			
median (years)	51	37	47
range (years)	27-77	22-75	19-87
Years of education (%)			
≤12 yrs	19	17	14
13-16 yrs	42	47	48
>16 yrs	39	36	38
Race (%)			
White, non-Hispanic	86	88	59
Non-white	14	12	41
Ashkenazi Jewish (%)	20	15	4
Current Body Mass Index (mg/kg^2			
Median	24.94	24.94	24.08
Smoking (%)			
Never	52	61	62
Former	42	33	23
Current	6	6	15
Alcohol Consumption per week (%)			
Never	27	24	22
<1	37	44	36
1-3	19	19	25
4-6	9	7	12
≥7	8	6	5
Age at menarche (%)			
≤11 yrs	20	22	21
12-13 yrs	62	51	52
≥14	18	27	27
Age at first birth (%)			
Nulliparous	18	22	50
<20 yrs	5	16	14
20-24 yrs	30	25	11
25-29 yrs	25	41	8
≥30 yrs	21	18	17
Breast fed (among parous women)			
Yes	61	55	40
Radiation exposure for (%)			
Acne treatment	5	2	1
Enlarged gland/tonsils	2	0	1
birth mark	0	1	0

Task 5: Enter questionnaire data.

Data from questionnaires and family history forms has been entered into our database.

Task 6: Analyze data and write report.

We anticipate completing analysis and writing of the final report in early 2002.

3. KEY RESEARCH ACCOMPLISHMENTS

A genotype-phenotype study was conducted using DNA from 31 women whose blood was assayed for DNA repair proficiency by the cytogenetic assay in our pilot study at NIH (2) (Appendix 1). We observed a strong association between presence of the Lys/Lys 751 XPD genotype, and higher number of chromatid aberrations in the cytogenetic assay (Table 2). XPD participates in nucleotide excision repair and transcription-coupled repair. Transcription-coupled repair affects repair of different types of DNA damage, including that due to ionizing radiation. There is genetic variation in XPD that results in amino acid substitutions: Asp312Asn, and Lys751Gln.

Table 2. Association of XPD genotypes with DNA repair proficiency

XPD Polymorphism	DNA Repair Proficiency[1]		
	Adequate	Suboptimal[2]	OR (95% CI)[3]
Asp312Asn			
Asp/Asn or Asn/Asn	46% (N=6)	31% (N=5)	1 (ref)
Asp/Asp	54% (N=7)	69% (N=11)	1.8 (0.3-11.0)
Lys751Gln			
Lys/Gln or Gln/Gln	73% (N=11)	25% (N=4)	1 (ref)
Lys/Lys	27% (N=4)	75% (N=12)	7.2 (1.01-87.7)

[1]Measured by examination of metaphase cells for chromatid breaks and gaps.
[2]Suboptimal repair: More than a total of 60 breaks and gaps per 100 metaphase cells. Distribution of breaks and gaps is bimodal, with two distinct and non-overlapping distributions.
[3]Age-adjusted
Adapted from Lunn et al., *Carcinogenesis* 21:551-555, 2000

We are currently extracting the DNA from the collected samples. Genotyping to determine common polymorphisms in candidate genes controlling enzymes in DNA repair pathways will be performed.

Characteristics of the two case groups and control group are shown in Table 2. Slightly more cases than controls had a history of radiation therapy. We are currently analyzing the differences between the group in exposure to medical x-rays (x-rays unrelated to the diagnosis of cancer). We will use this information to explore gene-environment interactions between

radiation exposure and presence of polymorphisms in DNA repair genes (e.g. XPD and XRCC1) and the risk of breast cancer. We expect to have this completed by February 2002.

4. REPORTABLE OUTCOMES:

Results of the genotype phenotype correlation were reported at the Symposium 20: Radiation Epidemiology: Radiosensitive Organs and Populations. Annual Meeting of the Radiation Research Society and North American Hyperthermia Society. Caribe Hilton, San Juan, Puerto Rico, April 21-26, 2001.

Genetic susceptibility in radiation epidemiology.
Kathy Helzlsouer[1], Emily Harris, Ruth Lunn, Katherine Sanford, Ram Parshad, Douglas Bell
[1]Johns Hopkins School of Hygiene and Public Health, Baltimore MD. USA

Suboptimal repair of DNA damage from environmental exposures such as ionizing radiation may predispose individuals to cancer. In a case-control study of women with a family history of breast cancer compared to low-risk control women, persistence of chromosomal damage (breaks + gaps >60) following irradiation to lymphocytes was assayed. measured. Women with a family history of breast cancer were much more likely than control women to have evidence of suboptimal repair of ionizing radiation-induced DNA damage (odds ratio=5.2, 95% confidence interval=1.04 -28.6). Subsequently we correlated the persistence of chromosomal damage following radiation with 3 polymorphisms in the XPD genotype. We observed a statistically significant association between the Lys/Lys 751 polymorphism of the XPD gene, a gene involved in nucleotide excision repair, and the presence of suboptimal DNA repair proficiency. The association between polymorphisms in XPD and other genes coding for enzymes involved in repair of DNA damage and subsequent development of breast and other cancers is being investigated.

Manuscript: Lunn RM, Helzlsouer KJ, Parshad R, Umbach DM, Harris EL, Sanford KK, Bell DA. XPD polymorphisms: effects on DNA repair proficiency. *Carcinogenesis* 2000; 21:551-555. (See Appendix 1).

Patents and licenses: not applicable

5. CONCLUSIONS:

We have identified a genotype-phenotype correlation between a polymorphism in XPD and a measure of DNA repair capacity (G2 lymphocyte irradiation assay). We will be examining the association between the polymorphism in this gene and other genes in DNA repair pathways and case status. Genotype results are expected by February 2002. These results have potential widespread implications in identifying susceptible populations of women who may be at higher risk of breast cancer due to low dose radiation exposures or other environmental exposures that cause DNA damage. A manuscript will be prepared once data are analyzed.

We learned the difficulties of transferring the G2 lymphocyte irradiation assay to another laboratory. These assays are difficult to do on a large scale basis. If common genetic

polymorphisms in genes encoding for DNA repair pathways can be identified that are associated with breast cancer risk, identification of susceptible populations can be done in an easier and more reliable manner.

List of Personnel:

Kathy J. Helzlsouer, M.D., M.H.S.	Principal Investigator
Emily L. Harris, Ph.D., M.P.H.	Co-Investigator
Nancy E. Davidson, M.D.	Co-Investigator
Helen Perry	Study Coordinator
Gloria Zepp	Secretary
Kim Butash	Lab Technician
Kathryn Linton, M.S.	Research Analyst/Kaiser Permanente

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2. Lunn RM, Helzlsouer KJ, Parshad R, Umbach DM, Harris EL, Sanford KK, Bell DA. XPD polymorphisms: effects on DNA repair proficiency. *Carcinogenesis* 2000; 21:551-555.

APPENDICES

Appendix 1: Lunn RM, Helzlsouer KJ, Parshad R, Umbach DM, Harris EL, Sanford KK, Bell DA. XPD polymorphisms: effects on DNA repair proficiency. Carcinogenesis 2000; 21:551-555.

Carcinogenesis vol.21 no.4 pp.551–555, 2000

ACCELERATED PAPER

XPD polymorphisms: effects on DNA repair proficiency

Ruth M.Lunn[1], Kathy J.Helzlsouer[2], Ram Parshad[3], David M.Umbach[4], Emily L.Harris[2,5], Katherine K.Sanford[6] and Douglas A.Bell[1,7]

[1]Laboratory of Computational Biology and Risk Analysis, National Institute of Environmental Health Sciences, National Institutes of Health, MD C3-03, PO Box 12233, Research Triangle Park, NC 27709, [2]Department of Epidemiology, The Johns Hopkins University School of Hygiene and Public Health, Baltimore, MD, [3]Department of Pathology, Howard University College of Medicine, Washington, DC, [4]Biostatistics Branch, National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, NC and [6]Laboratory of Cellular and Molecular Biology, Division of Cancer Etiology, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA

[5]Present address: Kaiser Permanente Center for Health Research, Portland, OR, USA

[7]To whom correspondence should be addressed
Email: bell1@niehs.nih.gov

***XPD* codes for a DNA helicase involved in transcription and nucleotide excision repair. Rare *XPD* mutations diminish nucleotide excision repair resulting in hypersensitivity to UV light and increased risk of skin cancer. Several polymorphisms in this gene have been identified but their impact on DNA repair is not known. We compared *XPD* genotypes at codons 312 and 751 with DNA repair proficiency in 31 women. *XPD* genotypes were measured by PCR–RFLP. DNA repair proficiency was assessed using a cytogenetic assay that detects X-ray induced chromatid aberrations (breaks and gaps). Chromatid aberrations were scored per 100 metaphase cells following incubation at 37°C (1.5 h after irradiation) to allow for repair of DNA damage. Individuals with the Lys/Lys codon 751 *XPD* genotype had a higher number of chromatid aberrations (132/100 metaphase cells) than those having a 751Gln allele (34/100 metaphase cells). Individuals having greater than 60 chromatid breaks plus gaps were categorized as having sub-optimal repair. Possessing a Lys/Lys751 genotype increased the risk of sub-optimal DNA repair (odds ratio = 7.2, 95% confidence interval = 1.01–87.7). The Asp312Asn XPD polymorphism did not appear to affect DNA repair proficiency. These results suggest that the Lys751 (common) allele may alter the XPD protein product resulting in sub-optimal repair of X-ray-induced DNA damage.**

Introduction

Hereditary genetic defects in DNA repair lead to increased risk of cancer. Individuals with xeroderma pigmentosum (XP), a rare autosomal recessive disease resulting from a defect in nucleotide excision repair (NER) of UV-damaged DNA, have a >1000-fold increased risk of skin cancer (1). These individuals are extremely hypersensitive to sunlight and have pigmentation abnormalities. Cell fusion analyses have identified seven genetic complementation groups (XPA to XPG) that encode for proteins participating in different steps of the NER pathway (2,3). NER deficiencies are also responsible for two other genetic diseases, Cockayne syndrome (CS), characterized by growth and mental retardation and neurological degeneration, and trichothiodystrophy (TTD), characterized by sulfur-deficient brittle hair and impaired mental and physical development (4,5).

Although CS, TTD and XP exhibit different clinical manifestations, they all have NER deficiencies. Moreover, all of these diseases can result from deficient XPD or XPB proteins. Recent elucidation of the functions of the various repair proteins encoded by the complementation groups has provided insight into these disease processes. NER is composed of two sub-pathways, global genome repair and transcription-coupled repair. Transcription-coupled repair occurs rapidly because it repairs damage to the transcribed strand of active genes, whereas global repair is much slower and repairs damage to inactive genes (6,7). XP results from defects in both transcription-coupled repair and global repair depending on the complementation group, and TTD and CS are caused by defective transcription-coupled repair and probably alterations in transcription (8). Transcription and repair are linked via the TFIIH complex, a basal transcription factor that participates in NER and transcription initiation. Moreover, XPB and XPD are components of the transcription factor, TFIIH, thus explaining their involvement in diseases with different phenotypes (9).

XPD protein possesses both single-strand DNA-dependent ATPase and 5′–3′ DNA helicase activities and is thought to participate in DNA unwinding during NER and transcription (9,10). NER repairs DNA damage induced by UV radiation and bulky DNA adducts. However, because XPD is involved in both transcription and NER, it may contribute to repair of other types of damage, such as ionizing radiation. Studies using lymphocytes containing mutant XP genes have an elevated chromatid aberration frequency after exposure to ionizing radiation, suggesting a role for NER proteins in the repair of ionizing radiation-induced damage (11). Ionizing radiation induces oxidative damage and several studies suggest XP proteins may participate in the repair of this type of damage (12,13).

Because XPD is important in multiple cellular tasks and rare *XPD* mutations result in genetic diseases, *XPD* polymorphisms may operate as genetic susceptibility factors. As a preliminary test of functionality, we studied the association of three *XPD* polymorphisms located at codons 199 (Ile→Met), 312 (Asp→Asn) and 751 (Lys→Gln) with proficiency for repair of X-ray-induced chromatid breaks and gaps. Reduced

Abbreviations: 95% CI, 95% confidence interval; CS, cockayne syndrome; NER, nucleotide excision repair; OR, odds ratio; TTD, trichothiodystrophy; XP, xeroderma pigmentosum.

Table I. PCR–RFLP: primers restriction enzymes and fragment sizes

	XPD (199)[a]	XPD (312)[a]	XPD (751)
PCR primers			
Forward	22872F: ctg ttg gtg ggt gcc cgt atc tgt tgg tct	22872F: ctg ttg gtg ggt gcc cgt atc tgt tgg tct	35844F: cct ctc cct ttc ctc tgt tc
Reverse	23952R: (mutant) taa tat cgg ggc tca ccc tgc agc act tcc t	23952: (mutant) taa tat cgg ggc tca ccc tgc agc act tcc t[b]	36560R: cag gtg agg ggg aca tct
PCR fragment (bp)	757	757	734
RFLP			
Restriction enzyme	*Dpn*II[c]	*Sty*I[c]	*Mbo*II
Fragment sizes (bp)			
Control cut	357	357	131
Wild-type homozygote	73, 176	151	98, 505
Heterozygote	73, 176, 243	34, 117, 151	98, 505, 603
Variant homozygote	243	34, 117	603
Agarose gel	3% 3:1 (NuSieve)	3% 3:1 (NuSieve)	2% 3:1 (NuSieve)

[a]Amino acids 199 and 312 amplified on same PCR fragment.
[b]taa tat added to the 5′ end of the primer; primer is mutated (g→c, underlined) at bp 23593.
[c]PCR fragment containing amino acids 199 and 312 was double digested with *Dpn*II and *Sty*I.

DNA repair as measured in this assay has been associated with a high degree of cancer incidence in family members (14–16). We found that the Lys/Lys751 was associated with reduced repair of X-ray-induced DNA damage.

Materials and methods

Subjects

We identified *XPD* genotypes and examined their association with DNA repair proficiency as measured previously in 31 Caucasian women from the Breast Surveillance Service at The Johns Hopkins Medical Institutions and female employees at the same institution (14). One woman had breast cancer, whereas the other women had no previous diagnosis of cancer and were categorized as either high risk (n = 15), defined as having at least one first-degree relative or two second-degree relatives on the same side of the family with breast cancer, or low risk (n = 15) for breast cancer.

DNA repair proficiency

DNA repair proficiency was assessed previously in a masked fashion using the assay developed by Sanford and co-workers (17,18). This assay measures unrepaired DNA (breaks and gaps) in cytogenetic preparations of metaphase lymphocytes isolated from freshly drawn blood of the subjects. The lymphocytes were exposed to X-rays and incubated to allow for repair. Colcemid was added after 0.5 h, the cells were then incubated for another 1 h and then lysed. Since the distribution of breaks and gaps is bimodal, individuals can be categorized as having normal (less than 60) and sub-optimal (greater than 60) repair proficiency based on the sum of breaks and gaps per 100 metaphase cells (14). Helzlsouer *et al.* (14) reported that sub-optimal DNA repair was more prevalent among women with a family history of breast cancer in this study population.

Genotyping

XPD genotypes were determined using a PCR–RFLP technique. Polymorphisms located at amino acids 199, 312 and 751, were amplified from 50 ng DNA using 200 μM of each dNTP, 0.5 U *Taq* (Promega, Madison, WI) + TaqStart Antibody (Sigma, St Louis, MO), 0.8 μM primer (Table I) and either 1.5 mM (codons 199 and 312) or 2.0 mM (codon 751) $MgCl_2$ in 1× PCR buffer (Promega). Codons 199 and 312 were amplified together using 5% DMSO as an additive, whereas codon 751 was amplified in a separate PCR reaction. Both reactions used the same PCR program which consisted of a 4 min denaturation step at 94°C followed by 30 cycles of 30 s at 94°C, 30 s at 60°C and 60 s at 72°C. The PCR amplicons were digested for 2 h at 37°C with a discriminating restriction enzyme, and the digestion products were separated by agarose gels. Table I delineates these conditions.

Statistical analysis

We compared the number of chromatid aberrations (per 100 metaphase cells) for each *XPD* genotype using the Mann–Whitney Rank Sum test. We also categorized individuals as having either normal or sub-optimal DNA repair proficiency as defined earlier and examined whether the risk of sub-optimal repair proficiency was associated with *XPD* genotype. We stratified by age (dichotomized at the mean age of 42 years for our subjects). Exact odds ratios (ORs) and 95% confidence intervals (95% CIs) were calculated via conditional maximum likelihood methods for 2×2×2 tables using LogXact software (Cytel Software Corporation, Cambridge, MA).

Results

We found no variants at codon 199 in our study population, precluding further analysis of that locus. The *XPD* 312 variant (Asn) allele occurred with a frequency of 0.42, similar to that reported by Shen *et al.* (19). The 751 variant (Gln) allele occurred with a frequency of 0.26 which is also comparable with previous reports (19,20) and to an independent North Carolina genotyping study conducted in our laboratory (unpublished results).

Mean and/or median chromatid breaks, gaps and totals (breaks + gaps) were calculated for each *XPD* genotype (Table II). Because variant homozygotes were rare (n = 2 and n = 1 for *XPD* 312 and 751, respectively), we combined them with heterozygotes and compared that pooled genotype with the more common homozygote genotype. Individuals homozygous for the Lys751 (common) allele had significantly (P = 0.01) higher chromatid aberrations (median = 132) than those having a Gln751 allele (median = 34). The median number of chromatid aberrations was also higher in individuals homozygous for the more common Asp312 allele than those having at least one variant Asn312 allele (132 and 50 breaks and gaps, respectively). However, this difference was not statistically significant (P = 0.22).

Figure 1 depicts chromatid aberrations plotted for each Lys751Gln genotype group and stratified by familial breast cancer risk status. Women having the Lys/Lys751 genotype had higher median chromatid aberrations than those with genotypes containing the variant Gln allele whether they came from the low-risk group (89 versus 34, respectively; P = 0.34) or from the high-risk group (136 versus 38, respectively; P = 0.05). While the difference is statistically significant only in the high-risk group, the number of individuals in each group is small. Interestingly, all the women (n = 7) having both the Lys/Lys751 genotype and categorized as having high risk for breast cancer had sub-optimal repair.

To assess the risk of sub-optimal repair due to different *XPD* alleles, we categorized individuals into two repair proficiency groups (sub-optimal and normal repair) based on the number of chromatid aberrations (Table III). In our study population, age was related to the Lys751Gln polymorphism (P = 0.01)

Table II. *XPD* genotypes and chromatid aberrations

XPD genotypes	*n*	Breaks [median (mean ± SEM)]	Gaps [median (mean ± SEM)]	Total (breaks+gaps) [median (mean ± SEM)]
Asp312Asn				
Asp/Asp	18	81 (62 ± 8.8)	46 (39 ± 5.7)	132[a] (100 ± 13.6)
Asp/Asn + Asn/Asn	12	34 (49 ± 9.41)	16 (28 ± 5.0)	50[a] (77 ± 14.9)
Lys751Gln				
Lys/Lys	16	82 (72 ± 8.0)	46 (42 ± 4.8)	132[c] (114 ± 12.5)
Lys/Gln + Gln/Gln	15	22 (36 ± 7.7)	14 (23 ± 4.7)	34[b] (59 ± 12.3)

[a]$P = 0.22$ by Mann–Whitney Rank Sum test.
[b]$P = 0.01$ by Mann–Whitney Rank Sum test.

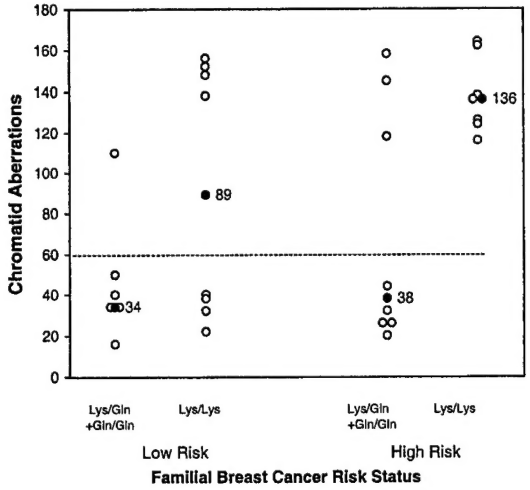

Fig. 1. *XPD* Lys751Gln genotypes in low and high breast cancer risk groups. Total chromatid aberrations (breaks and gaps) are plotted for *XPD* Lys751Gln genotypes (Lys/Lys and Lys/Gln + Gln/Gln) stratified according to familial breast cancer risk status. Open circles, data points for each individual; closed circles, median values for each group. The difference in chromatid aberrations between *XPD* genotypes, Lys/Lys versus Lys/Gln + Gln/Gln, stratified according to familial breast cancer risk was tested using the rank sum test ($P = 0.34$ and $P = 0.05$ for the low- and high-risk groups, respectively). The dotted line represents the bimodal division between good repair and sub-optimal repair (60 chromatid aberrations).

but not to risk status ($P = 0.6$) or to the Asn312Asp polymorphism ($P = 0.53$). We observed a significant increased risk of sub-optimal DNA repair for women having the Lys/Lys751 genotype (OR = 7.2, 95% CI = 1.01–87.7; $P = 0.035$), but not for the Asp/Asp312 genotype (OR = 1.8, 95% CI = 0.3–11.0, $P = 0.47$).

Discussion

In this study, the Lys/Lys751 genotype was associated with sub-optimal repair of DNA damage induced by X-irradiation. DNA repair was assessed using a cytogenetic assay that measures chromatid aberrations 0.5–1.5 h after treatment with X-rays. Chromatid aberrations result from unrepaired DNA strand breaks caused directly by X-irradiation, or indirectly as a result of repair of other X-ray-induced damage such as base damage (16). Persistence of these breaks suggests a deficiency in DNA repair. DNA repair proficiency, as measured by this cytogenetic assay, has been associated with predisposition to cancer (14–16). Parshad *et al.* (21) reported the sensitivity of the chromatid aberration assay was sufficient to detect XP carriers (heterozygotes) who were clinically normal. While the cytogenetic assay is well documented to detect deficient DNA repair, we need to address three points concerning our findings: (i) the relationship between X-ray irradiation and XP; (ii) possible discordance between the present work and results from previous studies of rare mutant *XPD* genotypes; and (iii) the high frequency of the 751 Lys allele (Lys/Lys751 is the most common genotype).

Table III. XPD genotypes and risk of sub-optimal DNA repair

XPD allele	Repair (good/sub-optimal)	OR; CI[a]
Asp312Asn		
Asp/Asn + Asn/Asn	6/5	1.0 (ref.)
Asp/Asp	7/11	1.8 (0.3–11.0); $P = 0.47$
Lys751Gln		
Lys/Gln + Gln/Gln	11/4	1.0 (ref.)
Lys/Lys	4/12	7.2 (1.01–87.7); $P = 0.035$

[a]Adjusted for age.

Clinically, XP is due to deficient NER repair of UV-induced damage. Nevertheless, XP mutant cell lines are deficient in the repair of ionizing radiation as measured by chromatid aberrations (11). XP proteins probably participate in the repair of oxidative damage induced by ionizing radiation. Satoh *et al.* (12) reported that extracts from XP cells were unable to repair a specific class of oxygen free radical induced base lesions. Oxidative damage induced by ionizing radiation is preferentially repaired on the transcribed strand, suggesting a role for enzymes such as XPD which participate in transcription-coupled repair (13). Thus, there is a plausible role for the XPD protein in the repair of some types of radiation-induced damage.

With regard to the second point, we observed an increased chromatid aberration frequency in cells from individuals having the Lys/Lys751 *XPD* polymorphism; however, Sanford *et al.* (22), using the same cytogenetic assay, reported that the chromatid aberration frequency was not elevated in cells containing a rare *XPD* mutation (obtained from an XP patient). This discrepancy may be due to the different location of the polymorphism and the mutation; the polymorphism occurs at codon 751 whereas most XP individuals in complementation group D have a mutation at codon 683, the putative nuclear location signal which is believed to be responsible for XP symptoms (8,23–25). XPD is part of the TFIIH transcription factor, which is a multi-protein complex involved in many different functions, including transcription, NER, transcription-coupled repair, apoptosis and cell cycle regulation (13). Thus, XPD interacts with many different proteins as part of this complex. Amino acid variants in different domains, such as 683 and 751, of XPD may affect different protein interactions, and result in the expression of different phenotypes. While we

have no additional data to support this hypothesis, it is possible that the 751 Lys allele could have different effects in different DNA repair pathways (as assessed using other DNA repair assays).

Thirdly, we unexpectedly found the more common allele (Lys751) was associated with higher levels of chromatid aberrations than the variant allele (Gln751). Dybdahl *et al.* (20) also reported that individuals with the common allele (Lys751) had an elevated, but not significantly so, risk of basal cell carcinoma (OR = 4.2, 95% CI = 0.8–24). While one might expect the common allele to confer protection rather than risk, putative 'at risk' genotypes of metabolism genes such as glutathione *S*-transferase M1 have frequencies in excess of 60% in some populations (26). In addition, the effect of a given allele on repair may depend on the exposure and interaction with other genes participating in DNA damage recognition, repair and cell cycle regulation.

We found that all of the individuals with both the Lys/Lys751 genotype and a family history of breast cancer had sub-optimal repair (data grouped in the upper right corner of Figure 1), as defined by an increase in chromosomal aberrations induced by G_2 X-irradiation. This chromosomal radiosensitivity has been shown to be associated with individuals reporting cancer, including breast cancer, in their family. Roberts *et al.* (27) studied cellular radiosensitivity, using a cytogenetic assay similar to ours, in family members of radiosensitive (breast cancer patients) and non-sensitive individuals. Segregation analysis suggested that the radiosensitivity was heritable with a single major gene accounting for 82% of the variance among family members. The addition of a second, rarer gene to the model resulted in a better fit of the data. The authors postulated that these cancer-predisposing genes were common, low penetrance alleles found in normal populations (27), such as polymorphisms in DNA repair genes. The present finding is consistent with the hypothesis that multiple low-penetrance alleles could be involved in heritable radiosensitivity. The *XPD* Lys/Lys751 polymorphisms, specifically, and underlying genetic determinants of familial risk, in general, would be possibilities. Because of the small study population (n = 31), however, further statistical analysis of possible inter-relationships among *XPD* genotype and familial risk status using the data available to us would not be informative. Also, the confidence intervals around the risk estimates are wide, indicating a need to confirm both the qualitative and quantitative results of this work by extending it to a much larger study population. Nevertheless, the finding that the Lys/Lys751 genotype is associated with sub-optimal repair provides sufficient evidence to justify further phenotype/genotype studies as well as determining the impact of the Lys751Gln polymorphism on cancer risk.

Acknowledgements

We would like to express our appreciation to Drs Harvey Mohrenweiser and Richard Shen (Lawrence Livermore National Laboratory) for sharing information about *XPD* polymorphisms. We also thank Drs Mariana Stern (NIEHS) and William Kaufmann (University of North Carolina, Chapel Hill) for critical review of the manuscript. This research was supported in part by Public Health Service (PHS) grant CA36390 from the National Cancer Institute, National Institutes of Health and Department of Defense grant DAMD 17-97-1-7072.

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Received December 14, 1999; revised February 7, 2000; accepted February 10, 2000